Closing the Gap between Antigenicity and Immunogenicity in HIV-1 Vaccine Design

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Neutralizing antibodies block the infection of cells–it is thus considered important to elicit them with vaccines. Many new concepts for broadly neutralizing-antibody-inducing vaccines for HIV are based on the molecular structure of the gp120 and gp41 Env proteins as antigens. These concepts are complicated by inconsistencies between the antigenic and immunogenic properties of key epitopes (e.g., b12 epitope). A key problem has been that HIV is extremely variable and employs a number of strategies to avoid being recognized by antibodies. This requires one to go beyond the molecular and antigenic structure of the Env proteins and consider the molecular determinants in infected individuals who mount potent, cross-reactive neutralizing antibody responses. However, considerably less is known about the molecular determinants of such immunogenicity and, consequently, it has been difficult to translate this information to an immunogen that elicits broadly neutralizing antibody. We sought to close this gap between antigenicity and immungenicity by identifying amino acid signatures in serum-derived Env genes that associate with broadly neutralizing antibody responses.

We developed and implemented computational strategies for identifying correlations between mutational patterns in the HIV-1 envelope glycoproteins (Env), gp120 and gp41, and neutralization phenotypes. Computational tools for robust clustering of like patterns of neutralization potency were designed based on K-means clustering, factoring in the uncertainty that results from limited sampling (bootstrap) and assay variability (noise). Phylogenetically corrected methods were used to identify associations between genetic mutations and distinct neutralization clusters. Viruses used in assays with HIV-1-positive serum samples were derived from sexually acquired infections and closely resembled transmitted/founder viruses. All utilized the chemokine receptor CCR5 and were considered to possess a tier-2 neutralization phenotype. Serum samples were obtained from HIV-1 chronically infected subjects who were enrolled in clinical protocols of the Center for HIV/AIDS Vaccine Immunology (CHAVI). The neutralization assay was measured as reductions in luciferase reporter gene expression after a single round of infection with Env-pseudotyped viruses.

We first applied these methods to the definition of mutations that correlated with susceptibility to the potent neutralizing antibody b12, as a means to explore the appropriateness of applying our computational strategies to neutralizing antibody phenotypes within the well-understood context of b12-gp120 interactions. Env sequences from 251 clonal viruses that were differentially sensitive to MAb b12 were analyzed. We identified ten b12-neutralization signatures, including seven either in the b12-binding surface of gp120 or in the V2 region of gp120 that have been previously shown to impact b12 sensitivity. Also, we provided possible mechanisms on how another three distal sites could impact b12 potency.

We then defined signatures in a panel of envelope glycoproteins sampled from HIV-1-infected individuals who made either potent or weak neutralizing antibody responses, with the hypothesis that common features of the envelope glycoproteins that elicit good antibodies in natural infection might be useful to incorporate as vaccine immunogens. A checkerboard-style neutralization dataset was developed, comprising a multi-subtype panel of clonal Env-pseudotyped viruses (n=25) and sera from 68 infected individuals from whom a serum gp160 sequence was derived by single genome amplification (SGA). Three distinct clusters of sera with high, medium, and low neutralization potencies were identified. By analysis of the SGA-derived Env sequences from these individuals, five out of six signature mutations were identified in the CD4-inducible (CD4i) region of gp120 that strongly associated with neutralization potency. This region forms the coreceptor binding site and facilitates HIV-1 entry into cells.

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Our findings suggest that the CD4i region of gp120 is a key determinant (although not necessarily a target) of broadly neutralizing antibody responses in HIV-1-infected individuals. In addition to providing new insights for immunogen design, these computational tools may be useful in delineating the neutralization epitopes of novel monoclonal antibodies.

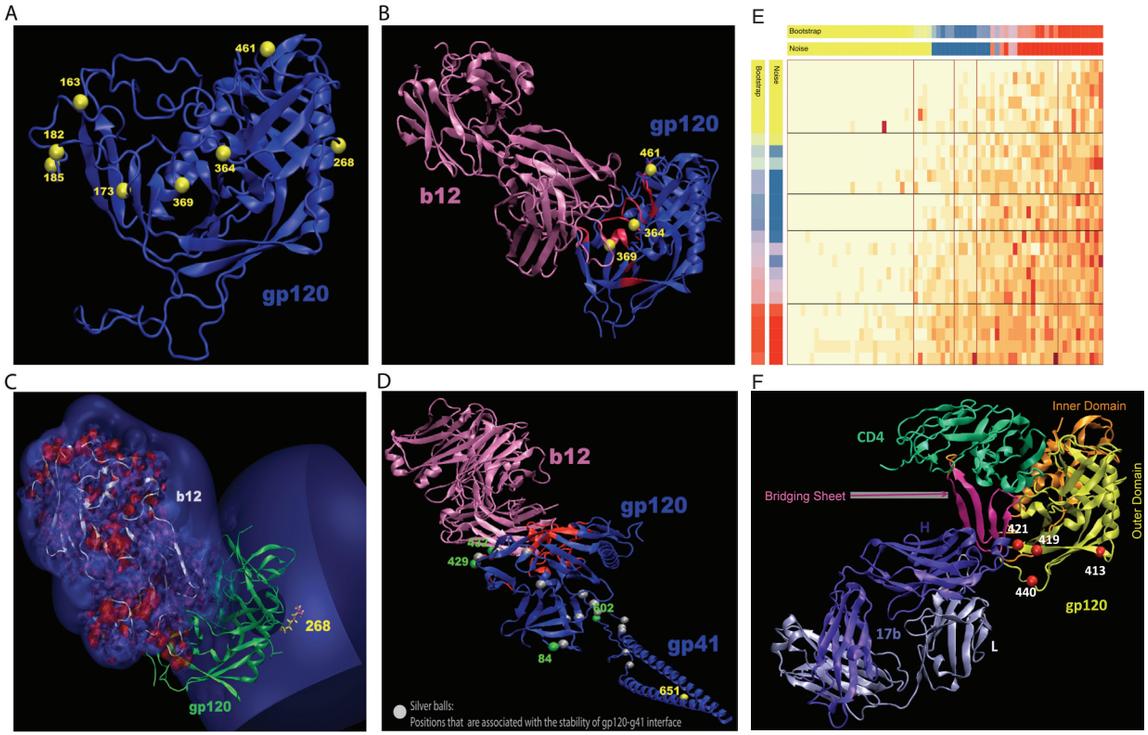

Fig.1. (A) b12 signature sites in a 3D structure of gp120. (B) Locations of three signature sites that occur at the b12 binding face of gp120. (C) Isosurface of the gp120 molecule showing the difference in electrostatic potential (+0.3 kT/e) due to mutation E268R in gp120, which results in a net positive electrostatic potential (blue) at the b12-gp120 interface region. (D) An illustration of position 651 could impact binding to b12 through an allosteric pathway involving the gp120-gp41 interface. (E) K-means clustering of serum samples and virus isolates in the test panel, k=3. Within the heat map, darker red indicates potent neutralization, progressively lighter colors through yellow indicate increasing resistance, and cream color is completely resistant. (F) The four signature sites in the CD4i region shown in a crystallographic 3D structure of gp120 complexed with CD4 and the CD4i-specific monoclonal antibody 17b.

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